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Novel approaches to combat bacterial biofilms

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Abstract

Biofilms formed by pathogenic bacteria and fungi are associated with a wide range of diseases, from device-related infections (such as catheters or prosthetic joints) to chronic infections occurring on native tissues (such as lung infections in cystic fibrosis patients). Biofilms are therefore responsible for an important medical and economic burden. Currently-used antibiotics have mostly been developed to target exponentially growing microorganisms and are poorly effective against biofilms. In particular, even high concentrations of bactericidal antibiotics are inactive against a subset of persistent biofilm bacteria, which can cause infection recurrence despite prolonged treatments. While the search for a magic bullet antibiotic effective against both planktonic and biofilm bacteria is still active, alternative preventive and curative approaches are currently being developed either limiting adhesion or biofilm formation or targeting biofilm tolerance by killing persister bacteria. Most of these approaches are adjunctive using new molecules in combination with antibiotics. This review presents promising approaches or strategies that could improve our ability to prevent or eradicate bacterial biofilms in medical settings.

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33 **Highlights**

- 34 • Currently-used antibiotics were developed to target planktonic bacteria
- 35 • Recent discoveries on biofilm properties led to promising anti-biofilm strategies
- 36 • Biofilm inhibition should integrate biocidal and non-biocidal approaches
- 37 • Jamming bacterial communication and regulation can prevent biofilm formation
- 38 • Major anti-biofilm approaches rely on matrix dissolution and potentiation of existing
- 39 antibiotics against persisters

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Introduction

Since the first observation of a direct link between development of biofilm and persistent infections [1-3], modern medicine is facing a double challenge: getting around the increasing concern of multidrug antibiotic resistance and tackling sources of biofilm-related infections. There is probably little hope to witness the rapid development of novel antibiotic molecules that would not only overcome multidrug resistance but also be more efficient than current antibiotics against medical biofilms. Indeed, most of the currently-used drugs have been developed and optimized to kill planktonic microorganisms.

The identification of novel molecules designed to specifically target mechanisms involved in biofilm formation or biofilm tolerance towards antibiotics could lead to novel therapies specifically designed to be combined with antibiotics against bacterial biofilm-associated infections. This review presents recent therapeutic approaches developed to specifically target biofilm-associated bacterial infections.

Strategies targeting specific mechanisms involved in biofilm formation

Anti-adhesion strategies

One of the crucial steps in biofilm development is the initial interaction of bacteria to abiotic or biotic surfaces that can ultimately lead to colonization and infection by pathogenic bacteria. Reducing adhesion is therefore a strategy of choice to prevent biofilm formation and related infections. Among the different strategies recently developed to reduce bacterial adhesion, one can distinguish strategies that non-specifically inhibit adhesion *versus* strategies that are rather targeting specific adhesins (**Figure**).

Non-specific inhibition of adhesion

Non-specific inhibition of adhesion is generally obtained by surface modification using polymers. The type of polymers can be chosen on the basis of its anti-adhesive properties. For example, Hook *et al.* assessed hundreds of polymeric materials using an high throughput microarray assay for their anti-adhesive properties and identified materials comprising ester and cyclic hydrocarbon moieties displaying anti-adhesive activity *in vitro* against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, and *in vivo*, against *S. aureus*, when grafted to silicone in a mice model of subcutaneously implanted device [4]. An efficient anti-adhesive molecule should not only limit bacterial proteins but also host proteins interaction to surfaces, therefore avoiding the formation of a conditioning film subsequently favoring bacterial colonization. Such molecules, for instance non-leaching polymeric sulfobetaine (polySB) that works as a wetting agent, have been demonstrated to reduce protein, host cells and microbial adhesion, but also thrombus formation *in vitro* and *in vivo* [5^{**}]. More recently, a biomimetic strategy using a glycocalyx-

like molecule, methyl-cellulose, displaying anti-adhesive property for both cells and bacteria, has been used to coat totally implanted venous access ports (TIVAP). Coated TIVAP implanted in rats for several days displayed important resistance toward adhesion, strongly reduced biofilm formation by *P. aeruginosa* and *S. aureus* as well as infective thrombus [6^{••}].

Beyond sole anti-adhesive strategies, surfaces combining different activities such as tissue integration, biocide property and anti-adhesive activity are currently developed [7[•]-9[•]]. A recent example of such a strategy displaying promising *in vitro* activity are anti-adhesive polymer brushes composed of block copolymer Pluronic F-127 (PF127) functionalized with antimicrobial peptides (AMP), able to kill bacteria on contact, and arginine-glycine-aspartate (RGD) peptides promoting the adhesion and spread of host tissue cells [10[•]].

Specific targeting of adhesins

Anti-biofilm approaches targeting specific adhesins have been shown to display strong anti-adhesive and anti-infective potential. Some molecules can impede the biogenesis of adhesins such as the one developed to block different fimbrial adhesins including the well-known type 1 fimbriae involved in bladder colonization by uropathogenic *E. coli* [11-14]. Type 1 fimbriae have also been the target of sugar analogues competing with eukaryotic receptors interacting with the tip-lectin, FimH. Among several molecules, some FimH inhibitors have been shown in mice to successfully prevent catheter-associated urinary tract infections by drug sensitive uropathogenic *E. coli* (UPEC) or to treat chronic cystitis in mice infected by the multidrug-resistant UPEC clone ST131 [15^{••},16[•]]. Carbohydrate inhibitors have also been developed against *P. aeruginosa* lectins, some of which prevented lung colonization in mouse models [17,18]. Interestingly, anti-biofilm action was

also achieved by using molecules combining several activities such as maltose derivatives with bulky hydro-carbon groups that presented both a surfactant/biofilm dispersion activity and an inhibition of adhesins/receptors mediated binding of *P. aeruginosa* [19].

Targeting biofilm maturation

Biofilm-related infection can also be reduced by blocking the biofilm maturation process (**Figure**). In most cases, strategies targeting biofilm maturation should also include treatment with an antimicrobial for an *in vivo* use to avoid the release of a massive quantity of biofilm bacteria into the bloodstream.

Major signaling pathways as antibiofilm targets

Among the major mechanisms that are governing biofilm maturation are quorum-sensing (QS) signals. We will not develop these aspects of anti-biofilm arsenal since several excellent reviews were recently written on the various strategies used to interfere with quorum-sensing including the use of analogues of homoserine lactones or AI-2 and enzymes degrading QS molecules for Gram-negative bacteria, and auto-inducing peptides or RNA-III inhibiting peptides for Gram-positive bacteria [20-22].

Discovery of the importance of small messenger molecule c-di-GMP in the physiological switch between planktonic to biofilm lifestyle is more recent and c-di-GMP is now considered as a valuable target to fight biofilm-related infections. Screening of chemical libraries led to the identification of direct or indirect inhibitors of diguanylate cyclases (the enzymes producing c-di-GMP), reducing biofilm formation such as sulfathiazole or azathioprine, an immunosuppressive drug [23-26]. Alternatively, a molecule impacting biofilm formation produced by *P. aeruginosa*, nitric oxide (NO), has been demonstrated to induce dispersal via the reduction of c-di-GMP concentrations through increased activity of

phospho-diesterases (PDE) [27], demonstrating the potential of compounds naturally produced by micro-organisms (see **Table**). In *P. aeruginosa*, NO-induced dispersal has been recently linked to a specific PDE, NdbA, whose mRNA transcription is induced by NO [28]. Interestingly, NO seems to be involved also in the dispersal of biofilms formed by other micro-organisms, however through a different mechanism involving H-NOX proteins. Therefore, development of surfaces releasing NO might be promising to control biofilm formation as demonstrated by the use of NO donor-coated urinary catheters and nanoparticles [29,30].

Direct action on matrix components to weaken biofilms

Two factors regulated by quorum-sensing and c-di-GMP play a major role in the architecture of biofilms: polysaccharides and extracellular DNA [31-34]. Thus, direct targeting of these factors instead of their signaling pathways can also be envisaged to reduce biofilm formation. Strategies using enzymatic degradation of these matrix components such as the use of DNaseI or Dispersin B, an hexosaminidase naturally produced by *Aggregatibacter actinomycetemcomitans* and hydrolyzing poly-N-acetylglucosamine (a frequent component of *E. coli*, *S. aureus* or *Staphylococcus epidermidis* exopolysaccharides), have been identified as efficient ways to disperse biofilms *in vitro* and *in vivo* (see for example, [35-37]). However, enzyme-based approaches are associated with two limitations: i) their restricted spectrum of action; and ii) the risk of immunization against these molecules. The association of chelators of divalent cations such as citrate or EDTA and biocides has also been proposed, based on their ability to destabilize biofilm matrix [38,39]. These chelators could find their interest in the case of local infection or restricted colonization such as device-related infection and have been used as preventive agents in clinical trials [40]. Additionally, EDTA was proven an efficient adjuvant to gentamicin to eradicate *E. coli*, *P. aeruginosa*, *S. aureus* and *S.*

epidermidis biofilms including persister cells (see below) in a rat model of catheter-related infection [41].

Strategies targeting mechanisms governing biofilm tolerance towards antibiotics: fighting persisters

One major problem caused by biofilms is their increased tolerance towards antimicrobial agents that impairs the treatment of biofilm-related infections in clinical settings [42]. While increased tolerance of biofilms is multifactorial, the main mechanism currently proposed to explain such tolerance is the presence of persisters, bacteria that enter in a specific phenotype state allowing them to survive in the presence of 1000 fold the minimum inhibitory concentration of bactericidal antibiotics [43,44]. Persister cells have recently been subjected to an intense hunt in order to limit biofilm-associated antibiotic tolerance.

Reducing persisters formation

There are now growing evidences that one of the main factors leading to persisters formation is nutritional stress, with a major effector molecule, ppGpp, the mediator of stringent response ([45], for a comprehensive review see [46]). Regarding the central role for ppGpp in persistence, it is tempting to hypothesize that strategies leading to reduced level of ppGpp could help fighting persisters. Relacin, a synthetic ppGpp analog inhibiting the *Bacillus subtilis* RelA synthetase activity and biofilm formation [47], and relacin derivatives displayed an inhibitory activity against different Rel proteins [48]. These different compounds still need to be assessed for their capacity to reduce persisters formation. Another stringent response inhibitor has been identified, the peptide 1018

(VRLIVAV- RIWRR-NH₂). While a direct evidence of its activity on persister cells is missing, this peptide displayed a specific antibiofilm activity against *in vitro* biofilms formed by several species including *P. aeruginosa* or *S. aureus* by inducing ppGpp degradation [49^{••}] and a synergistic action together with ciprofloxacin on *in vitro* biofilms of various pathogens [50[•]]. Interestingly, this latter study demonstrates how adjuvant therapies can allow reducing the concentration of antibiotic required to inhibit biofilm formation.

Killing persisters

Once a biofilm is mature, the last resort option for biofilm eradication is to identify compound that would increase antibiotic activity against persisters (**Figure**). Silver has been shown to potentiate the activity of several antibiotics against biofilm and persisters of Gram-negative and Gram-positive bacteria in a mouse biofilm model with subcutaneous catheter by increasing ROS production and bacterial permeability to antibiotics [51^{••}]. Sugar metabolism was also used to obtain antibiotic potentiation against persisters through an increased aminoglycosides penetration powered by the proton motive force [52,53]. Alcalinisation by basic amino-acids such as L-arginine was also recently demonstrated to enhance aminoglycoside action *in vitro* and *in vivo* against biofilms and persisters [54[•]]. Anti-QS molecules such as brominated furanones have the potential to revert antibiotic tolerance of *P. aeruginosa* or *E. coli* persister cells [55,56]. Persisters tolerance could also be reduced by exploiting their weaknesses related to their slow metabolism, such as a high sensitivity to proteolysis induced by the acyldepsipeptide ADEP4 that activates the ClpP protease in Gram-positive pathogens. ADEP4 is active in combination with rifampicin in a neutropenic mouse biofilm model [57^{••}]. Whereas the efficacy of these anti-persister approaches remains to be further validated, on-going

persister studies are likely to reveal other potential therapeutic strategies, such as the modulation of bacterial cell death [58].

Future perspectives

Much ado for almost nothing in clinic... Why?

The intense fundamental research on biofilms led to the emergence of numerous promising antibiofilm approaches. However, despite these long-lasting efforts, one should acknowledge that the translation of *in vitro* and *in vivo* data into clinical settings is slow and somewhat disappointing. Beyond the simple explanation of the massive costs necessary for drug development toward medical usage, one can identify potential reasons explaining this delay. Not only preventive strategies are difficult to translate into the clinic, but non-biocidal preventive anti-adhesive or anti-virulence strategies face the diversity of bacterial phenotypes and may only be active against a subpopulation of bacteria encountered in clinical settings, therefore limiting their overall efficacy. Then, even if *in vitro* biofilm susceptibility testing is a mandatory first step and much efforts have been made to develop such *in vitro* testing [59], molecules identified *in vitro* should be validated using relevant *in vivo* models for their antibiofilm activity but also absence of toxicity and pharmacokinetics.

The limitation of biofilm models

Despite the diversity of both the *in vitro* and the *in vivo* models currently available to identify or test antibiofilm molecules, *in vitro* models only partially reflect *in vivo* situations, because *in vitro* biofilms are probably structurally different and respond differently as

compared with *in vivo* biofilms [60]. In particular, antibiotic tolerance in biofilms has been obtained *in vitro* with starvation models [61] but other stress factors could also play a role in physiopathological conditions (flow, local pH, anoxia, inflammation...). At the present time, the diversity of persister phenotypes is not known, possibly due to the diversity of the *in vitro* conditions leading to persistence and the complexity of biofilm clinical situations. Efforts should therefore be made to enrich *in vitro* models with flow conditions, type of medium used, presence or absence of blood components or even specific eukaryotic cells within the device. These issues should also apply for *in vivo* models. Beyond the question of the relevance of using rodent models, some *in vivo* models may not properly reproduce real clinical situations. One can, for example, wonder about the relevance of the rat agar beads model to faithfully reproduce chronic infection in the lungs or subcutaneous model of catheters that are not connected to the bloodstream. Furthermore, as for clinical trials, rigorous statistical analysis and experimental set-up are mandatory in order to avoid any false positive interpretation. One can however foresee that increasing use of new guidelines for reporting animal research will also improve quality of experimental *in vivo* models [62,63].

What could be the near future?

Biofilm research will certainly benefit from the development of high throughput screenings evaluating compounds in combination with antibiotics using models better mimicking *in vivo* physiological conditions and new readouts benefiting from the increased knowledge on biofilm related signaling, such as reporter genes for pathways related to persistence (ppGpp, cyclic-di-GMP for Gram-negative bacteria, persisters metabolism). It is also expected that new formulations based on polymer microparticles could also emerge and

improve the use of otherwise topic compounds for local delivery at the site of biofilm infection [64,65]. To become more translational, biofilm research needs biomarkers and more global analyses performed directly on biofilms in clinical settings, which are currently essentially applied to *in vitro* or animal models. These omics analyses could provide new and unexpected targets. Lastly, the increasing awareness of the polymicrobial nature of biofilms should lead to the development of dedicated approaches to study bacteria-bacteria or bacteria-fungi interactions and their consequences on biofilm pathogenesis or tolerance towards antibiotics [66].

262 **Conflicts of interest**

263 All authors: no conflicts of interest.

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272

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Hoiby N: ***Pseudomonas aeruginosa* infection in cystic fibrosis. Diagnostic and prognostic significance of *Pseudomonas aeruginosa* precipitins determined by means of crossed immunoelectrophoresis. A survey.** *Acta Pathol Microbiol Scand Suppl* 1977;1-96.
2. Lam J, Chan R, Lam K, Costerton JW: **Production of mucoid microcolonies by *Pseudomonas aeruginosa* within infected lungs in cystic fibrosis.** *Infection and immunity* 1980, **28**:546-556.
3. Marrie TJ, Nelligan J, Costerton JW: **A scanning and transmission electron microscopic study of an infected endocardial pacemaker lead.** *Circulation* 1982, **66**:1339-1341.
4. Hook AL, Chang CY, Yang J, Luckett J, Cockayne A, Atkinson S, Mei Y, Bayston R, Irvine DJ, Langer R, et al.: **Combinatorial discovery of polymers resistant to bacterial attachment.** *Nature biotechnology* 2012, **30**:868-875.
5. Smith RS, Zhang Z, Bouchard M, Li J, Lapp HS, Brotske GR, Lucchino DL, Weaver D, Roth LA, Coury A, et al.: **Vascular catheters with a nonleaching poly-sulfobetaine surface modification reduce thrombus formation and microbial attachment.** *Science translational medicine* 2012, **4**:153ra132.
- This study demonstrated the efficacy of zwitterionic polymer surface modification to reduce, in several animal models of catheter-related infection, both thrombus and *E. coli* and *S. aureus* adhesion.
6. Chauhan A, Bernardin A, Mussard W, Kriegel I, Esteve M, Ghigo JM, Beloin C, Semetey V: **Preventing Biofilm Formation and Associated Occlusion by Biomimetic Glycocalyx-like Polymer in Central Venous Catheters.** *The Journal of infectious diseases* 2014, May 1. pii: jiu249. [Epub ahead of print].
- The first demonstration in a relevant *in vivo* model of catheter related infection of the efficacy of biomimetic antiadhesive polysaccharides fulfilling FDA requirement. *S. aureus* and *P. aeruginosa* biofilm formation is reduced by a factor of 10^4 to 10^5 fold reduction after 5 days of implantation.
7. Busscher HJ, van der Mei HC, Subbiahdoss G, Jutte PC, van den Dungen JJ, Zaat SA, Schultz MJ, Grainger DW: **Biomaterial-associated infection: locating the finish line in the race for the surface.** *Sci Transl Med* 2012, **4**:153rv110.
- A nice review on the development of material resistant to colonization insisting on the concept of combining grafted molecules with synergistic activities.
8. Campoccia D, Montanaro L, Arciola CR: **A review of the clinical implications of anti-infective biomaterials and infection-resistant surfaces.** *Biomaterials* 2013, **34**:8018-8029.
- A thorough review on all existing anti-infective biomaterials.
9. Hasan J, Crawford RJ, Ivanova EP: **Antibacterial surfaces: the quest for a new generation of biomaterials.** *Trends in biotechnology* 2013, **31**:295-304.
- A good review on surface modification techniques and discussion new generation of antibacterial surfaces, which are based on mimicking the surface nanotopography of natural surfaces.
10. Muszanska AK, Rochford ET, Gruszka A, Bastian AA, Busscher HJ, Norde W, van der Mei HC, Herrmann A: **Antiadhesive Polymer Brush Coating Functionalized with Antimicrobial and RGD Peptides to Reduce Biofilm Formation and Enhance Tissue Integration.** *Biomacromolecules* 2014, **15**:2019-2026.

- The authors reported a nice example of an anti-infective surface combining different molecules with a good antiadhesive and bactericidal properties without hampering tissue compatibility.
- 11. Cegelski L, Pinkner JS, Hammer ND, Cusumano CK, Hung CS, Chorell E, Aberg V, Walker JN, Seed PC, Almquist F, et al.: **Small-molecule inhibitors target *Escherichia coli* amyloid biogenesis and biofilm formation.** *Nat Chem Biol* 2009, **5**:913-919.
- 12. Lo AW, Van de Water K, Gane PJ, Chan AW, Steadman D, Stevens K, Selwood DL, Waksman G, Remaut H: **Suppression of type 1 pilus assembly in uropathogenic *Escherichia coli* by chemical inhibition of subunit polymerization.** *The Journal of antimicrobial chemotherapy* 2014, **69**:1017-1026.
- 13. Nait Chabane Y, Mlouka MB, Alexandre S, Nicol M, Marti S, Pestel-Caron M, Vila J, Jouenne T, De E: **Virstatin inhibits biofilm formation and motility of *Acinetobacter baumannii*.** *BMC microbiology* 2014, **14**:62.
- 14. Shamir ER, Warthan M, Brown SP, Nataro JP, Guerrant RL, Hoffman PS: **Nitazoxanide inhibits biofilm production and hemagglutination by enteroaggregative *Escherichia coli* strains by blocking assembly of AafA fimbriae.** *Antimicrobial agents and chemotherapy* 2010, **54**:1526-1533.
- 15. Totsika M, Kostakioti M, Hannan TJ, Upton M, Beatson SA, Janetka JW, Hultgren SJ, Schembri MA: **A FimH inhibitor prevents acute bladder infection and treats chronic cystitis caused by multidrug-resistant uropathogenic *Escherichia coli* ST131.** *The Journal of infectious diseases* 2013, **208**:921-928.
- The authors beautifully established the potential of orally administrated FimH inhibitors as an alternative treatment against multidrug-resistant *E. coli* using a mouse model of urinary tract infection.
- 16. Guiton PS, Cusumano CK, Kline KA, Dodson KW, Han Z, Janetka JW, Henderson JP, Caparon MG, Hultgren SJ: **Combinatorial small-molecule therapy prevents uropathogenic *Escherichia coli* catheter-associated urinary tract infections in mice.** *Antimicrob Agents Chemother* 2012, **56**:4738-4745.
- This study demonstrated the efficacy of combining anti-adhesive mannosides and trimethoprim-sulfamethoxazole to combat catheter-associated urinary tract infection in mice caused by *E. coli*.
- 17. Chemani C, Imberty A, de Bentzmann S, Pierre M, Wimmerova M, Guery BP, Faure K: **Role of LecA and LecB lectins in *Pseudomonas aeruginosa*-induced lung injury and effect of carbohydrate ligands.** *Infection and immunity* 2009, **77**:2065-2075.
- 18. Gening ML, Titov DV, Cecioni S, Audfray A, Gerbst AG, Tsvetkov YE, Krylov VB, Imberty A, Nifantiev NE, Vidal S: **Synthesis of multivalent carbohydrate-centered glycoclusters as nanomolar ligands of the bacterial lectin LecA from *Pseudomonas aeruginosa*.** *Chemistry* 2013, **19**:9272-9285.
- 19. Shetye GS, Singh N, Jia C, Nguyen CD, Wang G, Luk YY: **Specific Maltose Derivatives Modulate the Swarming Motility of Nonswarming Mutant and Inhibit Bacterial Adhesion and Biofilm Formation by *Pseudomonas aeruginosa*.** *Chembiochem : a European journal of chemical biology* 2014, **15**:1514-1523.
- The authors reported the *in vitro* activity of anti-adhesion and biofilm disruption activity of non-microbicidal disaccharide hydrocarbons displaying both a surfactant activity such as the one displayed by rhamnolipids and an inhibition of adhesion mediated by unknown *P. aeruginosa* adhesins.
- 20. Blackledge MS, Worthington RJ, Melander C: **Biologically inspired strategies for combating bacterial biofilms.** *Current opinion in pharmacology* 2013, **13**:699-706.
- 21. Rampioni G, Leoni L, Williams P: **The art of antibacterial warfare: Deception through interference with quorum sensing mediated communication.** *Biorganic Chemistry* 2014:1-9.
- 22. Zhu J, Kaufmann GF: **Quo vadis quorum quenching?** *Current Opinion in Pharmacology* 2013, **13**:688-698.

23. Sambanthamoorthy K, Luo C, Pattabiraman N, Feng X, Koestler B, Waters CM, Palys TJ: **Identification of small molecules inhibiting diguanylate cyclases to control bacterial biofilm development.** *Biofouling* 2014, **30**:17-28.
24. Sambanthamoorthy K, Sloup RE, Parashar V, Smith JM, Kim EE, Semmelhack MF, Neiditch MB, Waters CM: **Identification of small molecules that antagonize diguanylate cyclase enzymes to inhibit biofilm formation.** *Antimicrobial agents and chemotherapy* 2012, **56**:5202-5211.
25. Antoniani D, Rossi E, Rinaldo S, Bocci P, Lolicato M, Paiardini A, Raffaelli N, Cutruzzola F, Landini P: **The immunosuppressive drug azathioprine inhibits biosynthesis of the bacterial signal molecule cyclic-di-GMP by interfering with intracellular nucleotide pool availability.** *Applied microbiology and biotechnology* 2013, **97**:7325-7336.
26. Antoniani D, Bocci P, Maciag A, Raffaelli N, Landini P: **Monitoring of diguanylate cyclase activity and of cyclic-di-GMP biosynthesis by whole-cell assays suitable for high-throughput screening of biofilm inhibitors.** *Appl Microbiol Biotechnol* 2010, **85**:1095-1104.
27. Barraud N, Schleheck D, Klebensberger J, Webb JS, Hassett DJ, Rice SA, Kjelleberg S: **Nitric oxide signaling in *Pseudomonas aeruginosa* biofilms mediates phosphodiesterase activity, decreased cyclic di-GMP levels, and enhanced dispersal.** *J Bacteriol* 2009, **191**:7333-7342.
28. Li Y, Heine S, Entian M, Sauer K, Frankenberg-Dinkel N: **NO-induced biofilm dispersion in *Pseudomonas aeruginosa* is mediated by an MHYT domain-coupled phosphodiesterase.** *Journal of bacteriology* 2013, **195**:3531-3542.
29. Kishikawa H, Ebberyd A, Romling U, Brauner A, Luthje P, Lundberg JO, Weitzberg E: **Control of pathogen growth and biofilm formation using a urinary catheter that releases antimicrobial nitrogen oxides.** *Free radical biology & medicine* 2013, **65**:1257-1264.
30. Duong HT, Jung K, Kutty SK, Agustina S, Adnan NN, Basuki JS, Kumar N, Davis TP, Barraud N, Boyer C: **Nanoparticle (Star Polymer) Delivery of Nitric Oxide Effectively Negates *Pseudomonas aeruginosa* Biofilm Formation.** *Biomacromolecules* 2014.
31. Fazli M, Almblad H, Rybtke ML, Givskov M, Eberl L, Tolker-Nielsen T: **Regulation of biofilm formation in *Pseudomonas* and *Burkholderia* species.** *Environmental microbiology* 2014, **16**:1961-1981.
32. Ueda A, Wood TK: **Tyrosine Phosphatase TpbA of *Pseudomonas aeruginosa* Controls Extracellular DNA via Cyclic Diguanylic Acid Concentrations.** *Environmental microbiology* 2010, **2**:449-455.
33. Gloag ES, Turnbull L, Huang A, Vallotton P, Wang H, Nolan LM, Mililli L, Hunt C, Lu J, Osvath SR, et al.: **Self-organization of bacterial biofilms is facilitated by extracellular DNA.** *Proceedings of the National Academy of Sciences of the United States of America* 2013, **110**:11541-11546.
34. Flemming HC, Wingender J: **The biofilm matrix.** *Nature reviews. Microbiology* 2010, **8**:623-633.
35. Kaplan JB, Velliyagounder K, Ragunath C, Rohde H, Mack D, Knobloch JK, Ramasubbu N: **Genes involved in the synthesis and degradation of matrix polysaccharide in *Actinobacillus actinomycetemcomitans* and *Actinobacillus pleuropneumoniae* biofilms.** *Journal of bacteriology* 2004, **186**:8213-8220.
36. Hymes SR, Randis TM, Sun TY, Ratner AJ: **DNase inhibits *Gardnerella vaginalis* biofilms *in vitro* and *in vivo*.** *J Infect Dis* 2013, **207**:1491-1497.
37. Darouiche RO, Mansouri MD, Gawande PV, Madhyastha S: **Antimicrobial and antibiofilm efficacy of triclosan and DispersinB combination.** *J Antimicrob Chemother* 2009, **64**:88-93.
38. Banin E, Brady KM, Greenberg EP: **Chelator-induced dispersal and killing of *Pseudomonas aeruginosa* cells in a biofilm.** *Appl Environ Microbiol* 2006, **72**:2064-2069.
39. Turakhia MH, Cooksey KE, Characklis WG: **Influence of a calcium-specific chelant on biofilm removal.** *Appl Environ Microbiol* 1983, **46**:1236-1238.
40. Ferreira Chacon JM, Hato de Almeida E, de Lourdes Simoes R, Lazzarin COV, Alves BC, Mello de Andrea ML, Santiago Biernat M, Biernat JC: **Randomized study of minocycline and edetic acid as a locking solution for central line (port-a-cath) in children with cancer.** *Chemotherapy* 2011, **57**:285-291.

41. Chauhan A, Lebeaux D, Ghigo JM, Beloin C: **Full and broad-spectrum *in vivo* eradication of catheter-associated biofilms using gentamicin-EDTA antibiotic lock therapy.** *Antimicrob Agents Chemother* 2012, **56**:6310-18.
 - This study described the first formal demonstration of the strong adjunctive activity of the chelator EDTA *in vivo* as a curative therapy of catheter colonization when combined with gentamicin. A single-dose treatment with a gentamicin-EDTA lock solution fully eradicated both Gram-positive and Gram-negative bacterial biofilms formed in totally implanted venous access ports in rats.
42. Lebeaux D, Ghigo JM, Beloin C: **Biofilm-related infections: bridging the gap between clinical management and fundamental aspects of recalcitrance towards antibiotics.** *Microbiology and Molecular Biology Reviews* 2014, In press.
43. Maisonneuve E, Gerdes K: **Molecular Mechanisms Underlying Bacterial Persisters.** *Cell* 2014, **157**:539-548.
44. Zhang Y: **Persisters, persistent infections and the Yin–Yang model.** *Emerging Microbes & Infections* 2014, **3**:e3.
45. Nguyen D, Joshi-Datar A, Lepine F, Bauerle E, Olakanmi O, Beer K, McKay G, Siehnel R, Schafhauser J, Wang Y, et al.: **Active starvation responses mediate antibiotic tolerance in biofilms and nutrient-limited bacteria.** *Science* 2011, **334**:982-986.
46. Amato SM, Fazen CH, Henry TC, Mok WW, Orman MA, Sandvik EL, Volzing KG, Brynildsen MP: **The role of metabolism in bacterial persistence.** *Frontiers in microbiology* 2014, **5**:70.
 - A very interesting review gathering information demonstrating the pivotal role of metabolism in the entry, maintenance, and exit from the persister phenotype. This review also highlights the successes and potential of targeting metabolism in the search for anti-persister therapies, and discuss the current methods and challenges to understand persister physiology.
47. Wexselblatt E, Oppenheimer-Shaanan Y, Kaspy I, London N, Schueler-Furman O, Yavin E, Glaser G, Katzhendler J, Ben-Yehuda S: **Relacin, a novel antibacterial agent targeting the Stringent Response.** *PLoS pathogens* 2012, **8**:e1002925.
48. Wexselblatt E, Kaspy I, Glaser G, Katzhendler J, Yavin E: **Design, synthesis and structure–activity relationship of novel Relacin analogs as inhibitors of Rel proteins.** *European Journal of Medicinal Chemistry* 2013, **70**:497-504.
49. de la Fuente-Núñez C, Reffuveille F, Haney EF, Straus SK, Hancock REW: **Broad-Spectrum Anti-biofilm Peptide That Targets a Cellular Stress Response.** *PLoS pathogens* 2014, **10**:e1004152.
 - The very first demonstration of a molecule, here the peptide 1018, targeting stringent response mediator ppGpp that can negatively impact on biofilm development and maintenance.
50. Reffuveille F, de la Fuente-Nunez C, Mansour S, Hancock RE: **A broad-spectrum anti-biofilm peptide enhances antibiotic action against bacterial biofilms.** *Antimicrobial agents and chemotherapy* 2014, Jun 30. pii: AAC.03163-14. [Epub ahead of print].
 - This study described the synergistic interactions of different antibiotics with the anti-biofilm peptide 1018 to potentially prevent and eradicate *in vitro* bacterial biofilms formed by various multidrug-resistant microorganisms such as *P. aeruginosa*, *E. coli*, *A. baumannii*, *K. pneumoniae*, *S. enterica* and methicillin-resistant *S. aureus*.
51. Morones-Ramirez JR, Winkler JA, Spina CS, Collins JJ: **Silver enhances antibiotic activity against gram-negative bacteria.** *Science translational medicine* 2013, **5**:190ra181.
 - The authors nicely demonstrated how the activity of silver can lead to ROS production and enhanced membrane permeability and harnessed this effect to show that silver can potentiate bactericidal antibiotics against *in vitro* and *in vivo* biofilm formed by *E. coli*.
52. Allison KR, Brynildsen MP, Collins JJ: **Metabolite-enabled eradication of bacterial persisters by aminoglycosides.** *Nature* 2011, **473**:216-220.

53. Barraud N, Buson A, Jarolimek W, Rice SA: **Mannitol enhances antibiotic sensitivity of persister bacteria in *Pseudomonas aeruginosa* biofilms.** *PloS one* 2013, **8**:e84220.
54. Lebeaux D, Chauhan A, Letoffe S, Fischer F, de Reuse H, Beloin C, Ghigo JM: **pH-mediated potentiation of aminoglycosides kills bacterial persisters and eradicates *in vivo* biofilms.** *The Journal of infectious diseases* 2014, May 15. pii: jiu286. [Epub ahead of print].
 - This study reported that raising pH using basic clinically compatible amino-acids such as L-arginine can potentiate the activity of aminoglycosides against persisters and can allow the eradication of *E. coli* and *S. aureus* biofilms formed in totally implanted venous access ports in rats.
55. Pan J, Ren D: **Structural effects on persister control by brominated furanones.** *Bioorganic & medicinal chemistry letters* 2013, **23**:6559-6562.
56. Pan J, Xie X, Tian W, Bahar AA, Lin N, Song F, An J, Ren D: **(Z)-4-bromo-5-(bromomethylene)-3-methylfuran-2(5H)-one sensitizes *Escherichia coli* persister cells to antibiotics.** *Applied microbiology and biotechnology* 2013, **97**:9145-9154.
57. Conlon BP, Nakayasu ES, Fleck LE, LaFleur MD, Isabella VM, Coleman K, Leonard SN, Smith RD, Adkins JN, Lewis K: **Activated ClpP kills persisters and eradicates a chronic biofilm infection.** *Nature* 2013, **503**:365-370.
 - An elegant demonstration that activation and corruption of a target enzymatic activity can allow killing dormant persisters. The authors established that combining the antibiotic ADEP4 that activates ClpP with rifampicin led to complete eradication of *S. aureus* biofilms *in vitro* and in a mouse model of a chronic infection.
58. Thomas VC, Sadykov MR, Chaudhari SS, Jones J, Endres JL, Widhelm TJ, Ahn JS, Jawa RS, Zimmerman MC, Bayles KW: **A central role for carbon-overflow pathways in the modulation of bacterial cell death.** *PLoS pathogens* 2014, **10**:e1004205.
 - The authors demonstrated that the interplay between two metabolic enzymes modulate cell death and achieve optimal biofilm biomass in *S. aureus*, and that disturbing this process can lead to a reduced colonization in a rabbit model of endocarditis.
59. Macia MD, Rojo-Molinero E, Oliver A: **Antimicrobial susceptibility testing in biofilm-growing bacteria.** *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases* 2014, Apr 26. [Epub ahead of print]
60. Lebeaux D, Chauhan A, Rendueles O, Beloin C: **From *in vitro* to *in vivo* Models of Bacterial Biofilm-Related Infections.** *Pathogens* 2013, **2**:288-356.
 - A very thorough review describing all existing *in vitro* and *in vivo* existing models developed to study biofilm formation and the contribution of these models in a better understanding of biofilm physiology and the design of future efficient antibiofilm strategies.
61. Bernier SP, Lebeaux D, DeFrancesco AS, Valomon A, Soubigou G, Coppee JY, Ghigo JM, Beloin C: **Starvation, together with the SOS response, mediates high biofilm-specific tolerance to the fluoroquinolone ofloxacin.** *PLoS genetics* 2013, **9**:e1003144.
 - The authors established using a transposon mutants library of biofilm forming *E. coli* that amino-acids or carbon source starvation and the SOS response can strongly increase persisters levels specifically in biofilms.
62. Bolker J: **Model organisms: There's more to life than rats and flies.** *Nature* 2012, **491**:31-33.
63. Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG: **Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research.** *PLoS biology* 2010, **8**:e1000412.
64. Forier K, Raemdonck K, De Smedt SC, Demeester J, Coenye T, Braeckmans K: **Lipid and polymer nanoparticles for drug delivery to bacterial biofilms.** *Journal of controlled release : official journal of the Controlled Release Society* 2014, Apr 30. pii: S0168-3659(14)00266-1. [Epub ahead of print]
65. Miller KG, Tran PL, Haley CL, Kruzek C, Colmer-Hamood JA, Myntti M, Hamood AN: **Next Science Wound Gel Technology, a Novel Agent That Inhibits Biofilm Development by**

- Gram-Positive and Gram-Negative Wound Pathogens.** *Antimicrobial agents and chemotherapy* 2014, **58**:3060-3072.
- A nice demonstration of the efficacy of a newly developed and protected gel formulation that is capable when applied topically to strongly inhibit biofilm formation of *S. aureus* and *P. aeruginosa* in a murine model of wound infection.
- Burmolle M, Ren D, Bjarnsholt T, Sorensen SJ: **Interactions in multispecies biofilms: do they actually matter?** *Trends in microbiology* 2014, **22**:84-91.
 - Paredes J, Alonso-Arce M, Schmidt C, Valderas D, Sedano B, Legarda J, Arizti F, Gomez E, Aguinaga A, Del Pozo JL, et al.: **Smart central venous port for early detection of bacterial biofilm related infections.** *Biomedical microdevices* 2014, **16**:365-374.
 - Gil C, Solano C, Burgui S, Latasa C, Garcia B, Toledo-Arana A, Lasa I, Valle J: **Biofilm matrix exoproteins induce a protective immune response against *Staphylococcus aureus* biofilm infection.** *Infection and immunity* 2014, **82**:1017-1029.
 - Pinkston KL, Singh KV, Gao P, Wilganowski N, Robinson H, Ghosh S, Azhdarinia A, Seveck-Muraca EM, Murray BE, Harvey BR: **Targeting pili in enterococcal pathogenesis.** *Infection and immunity* 2014, **82**:1540-1547.
 - May RM, Hoffman MG, Sogo MJ, Parker AE, O'Toole GA, Brennan AB, Reddy ST: **Micro-patterned surfaces reduce bacterial colonization and biofilm formation *in vitro*: Potential for enhancing endotracheal tube designs.** *Clinical and translational medicine* 2014, **3**:8.
 - Weng L, Zhang Y, Yang Y, Wang L: **Isolation of the autoinducer-quenching strain that inhibits LasR in *Pseudomonas aeruginosa*.** *International journal of molecular sciences* 2014, **15**:6328-6342.
 - Pei R, Lamas-Samanamud GR: **Inhibition of biofilm formation by T7 bacteriophages producing quorum quenching enzymes.** *Applied and environmental microbiology* 2014, Jun 20. pii: AEM.01434-14. [Epub ahead of print]
 - Singh PK, Donovan DM, Kumar A: **Intravitreal injection of the chimeric phage endolysin Ply187 protects mice from *Staphylococcus aureus* endophthalmitis.** *Antimicrobial agents and chemotherapy* 2014, Jun 2. pii: AAC.00126-14. [Epub ahead of print].
 - Monnappa AK, Dwidar M, Seo JK, Hur JH, Mitchell RJ: ***Bdellovibrio bacteriovorus* inhibits *Staphylococcus aureus* biofilm formation and invasion into human epithelial cells.** *Scientific reports* 2014, **4**:3811.
 - Lu Y, Slomberg DL, Schoenfisch MH: **Nitric oxide-releasing chitosan oligosaccharides as antibacterial agents.** *Biomaterials* 2014, **35**:1716-1724.
 - Zhang A, Mu H, Zhang W, Cui G, Zhu J, Duan J: **Chitosan Coupling Makes Microbial Biofilms Susceptible to Antibiotics.** *Scientific Reports* 2013, **3**:3364.

Table 1. Recent studies illustrating some promising non-pharmaceutical anti-biofilm strategies. This list is not comprehensive and is only meant to illustrate each approach.

Mode of action	<i>In vitro</i>	<i>In vivo</i>	References
<i>Early detection</i>			
Detection of biofilm formation in a central venous catheter (CVC) using impedimetric biosensor	Detection of <i>S. epidermidis</i> biofilm formation within the chamber of a CVC	-	[67]
<i>Vaccination</i>			
Immunization against Biofilm Matrix Exoproteins from <i>S. aureus</i>	-	Reduction of <i>S. aureus</i> biofilm formation in a mesh biofilm model in mice	[68]
Passive protection with a monoclonal antibody against <i>Enterococcus faecalis</i> major pili protein EbpC	Prevention of <i>E. faecalis</i> biofilm formation	Significant passive protection against <i>E. faecalis</i> endocarditis in a rat model	[69]
<i>Inhibition of microbial adhesion</i>			
Modification of physical architecture of the surface (Sharklet micropattern)	Reduction of <i>E. coli</i> , <i>P. aeruginosa</i> , <i>A. baumannii</i> and <i>K. pneumonia</i> adhesion	-	[70]
<i>Bio-inspired strategies</i>			
Quorum-sensing quencher New quorum-sensing quencher (F5, LasR inhibitor) from	Reduction of <i>P. aeruginosa</i> biofilm	-	[71]

T7 engineered lytic phage producing a lactonase	Inhibition of mixed <i>P. aeruginosa</i> and <i>E. coli</i> biofilm	-	[72]
<hr/>			
Lytic enzymes from predators			
Chimeric phage endolysin degrading peptidoglycan	Disruption of <i>S. aureus</i> preformed biofilm	Attenuation of <i>S. aureus</i> mediated endophthalmitis in mice	[73]
<i>Bdellovibrio bacteriovorus</i> proteases and DNase	Prevention of <i>S. aureus</i> biofilm formation and disruption of <i>S. aureus</i> preformed biofilm	-	[74]
<hr/>			
Other activity			
Chitosan coupling with antibiotic or nitric oxide	Disruption of and inhibition of <i>Listeria</i> , <i>E. faecalis</i> and <i>S. aureus</i> biofilm by chitosan-streptomycine conjugate ; disruption of <i>P. aeruginosa</i> biofilms by chitosan-NO conjugate	-	[75,76]
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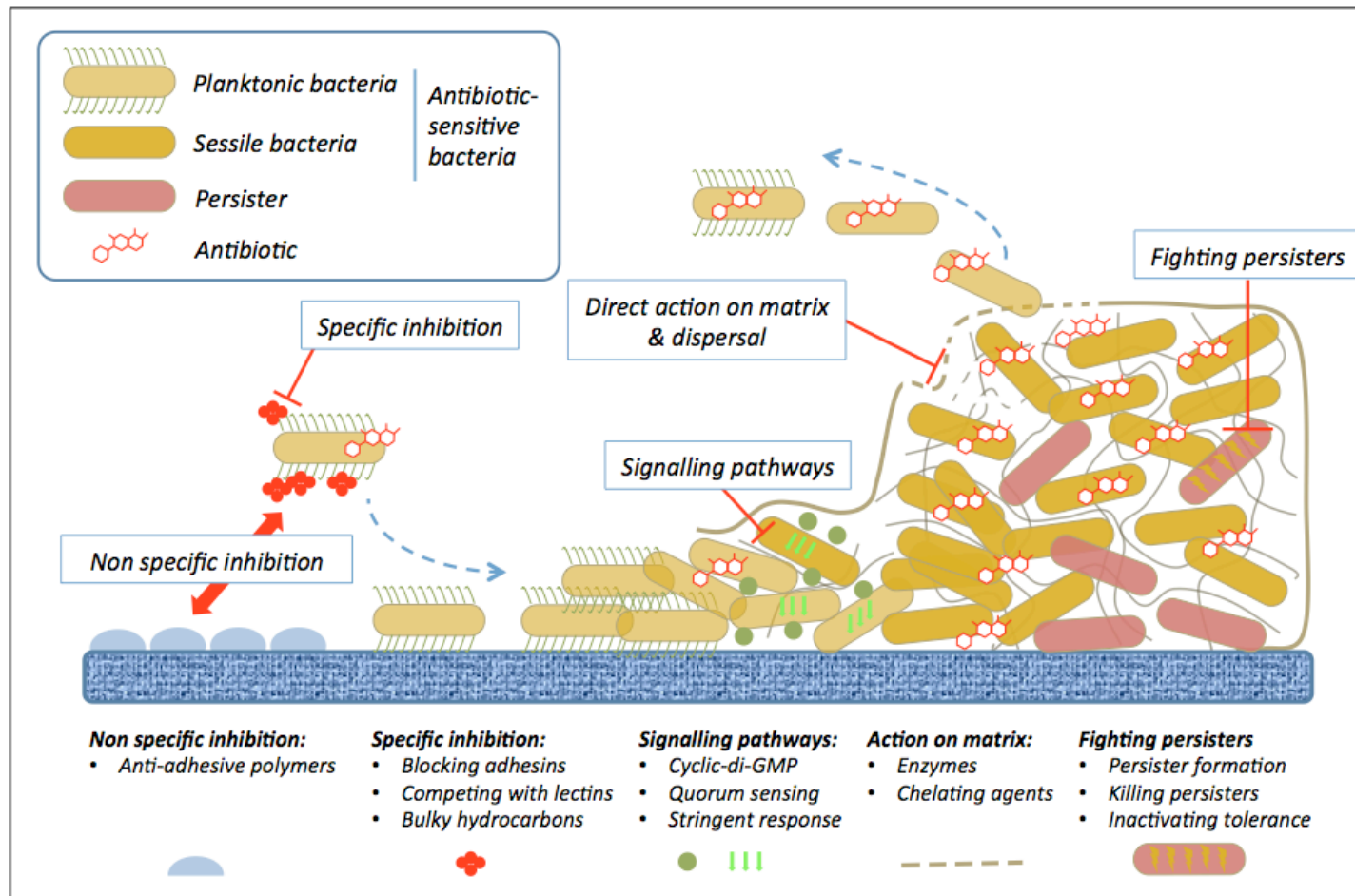


Figure 1. Biofilms: bacterial phenotypes and therapeutic targets

Schematic drawing of the successive steps of biofilm formation and maturation highlighting the different bacterial phenotypes encountered and their susceptibility to antibiotics. The five major approaches to combat biofilms are represented with their impact on biofilm formation or integrity and their possible combination with antibiotics.